Description and variation of a new Brazilian species of the *Hyla rubicundula* group (Anura, Hylidae)

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A new member of the *Hyla rubicundula* species group is described from Bela Vista, Mato Grosso do Sul, Brazil. The new species inhabits "cerrado" formations in central and southeastern Brazil, comprising three main geographic morphotypes. Intrapopulation and interpopulation variation is characterized in order to accurately determine the species limits. The analysis was based on external morphology and morphometrics. The new *Hyla* is characterized by being the largest in the *Hyla rubicundula* species group, having a stout body, lateral limits of dorsum under the lower border of the tympanum, immaculate dorsum, and rounded canthus rostralis.

INTRODUCTION

The *Hyla rubicundula* species group was defined by NAPOLI & CARAMASCHI (1998, 1999) and is known to comprise six species: *H. rubicundula* Reinhardt & Lütken, 1862; *H. trítaeniata* Bokermann, 1965, *H. anataliasiasi* Bokermann, 1972; *H. araguaya* Napoli & Caramaschi, 1998; *H. cerradensis* Napoli & Caramaschi, 1998, and *H. cachimbo* Napoli & Caramaschi, 1999.

Hyla rubicundula has the widest geographic distribution of all members of the group, occurring in southeastern, northeastern and central Brazil (NAPOLI & CARAMASCHI, 1999). Specimens from the Brazilian states of Mato Grosso, Mato Grosso do Sul and São Paulo are easily diagnosed from the topotypic samples (Lagoa Santa, Minas Gerais) by their greater size, robust body, distinct head shape, lateral limits of dorsum under the lower border of tympanum, and immaculate dorsum. These diagnostic characters suggest the presence of a new species.

Herein we describe a new species of the *Hyla rubicundula* group and report on intra- and interpopulation variation of this new species, in order to furnish accurate species limits.

Material and methods

Specimens used for descriptions or examined for comparisons are deposited in the following Brazilian collections. Célio F. B. Haddad Collection, deposited in the Departamento de Zoologia, Universidade Estadual Paulista, Rio Claro (CFBH), Departamento de Zoologia, Universidade Estadual Paulista, São José do Rio Preto (DZSJRP); Eugenio Izecksohn Collection, deposited in the Departamento de Biologia Animal, Universidade Federal Rural do Rio de Janeiro (EI); Jorge Jim Collection, deposited in the Departamento de Zoologia, Universidade Estadual Paulista, Botucatu (JJ), Museu Nacional, Rio de Janeiro (MNRJ); Museu de Zoologia, Universidade de São Paulo (MZUSP); Museu de História Natural, Universidade Estadual de Campinas (ZUEC).

We examined only adult males because of the rareness of females and juveniles in the samples. Larvae and advertisement calls are unknown. The specimens were sorted into morphotypes by similar morphology and proximity of localities. The measurements used in the account follow Napoli & Caramaschi (1998, 1999) and are in millimeters: SVL (snout-vent length), HL (head length), HW (head width), ED (eye diameter), UEW (upper eyelid width); IOD (interorbital distance); IND (internarial distance); TD (tympanum diameter), END (eye to nostril distance); NSD (nostril to tip of snout distance), UAR (upper arm); FAR (forearm); HAL (hand length); 3FD (third finger disk diameter), THL (thigh length); TL (tibia length); FL (foot length), and 4TD (fourth toe disk diameter). Webbing formula notation follows Savage & Heyer (1967) as modified by Myers & Duellman (1982).

Discriminant function analysis was used to analyze inter-population variation (Marcus, 1990), but without applying any method to remove the size effect in the groups (Reis et al., 1990). The groups under study were defined a priori, and the eigenvectors and their associated eigenvalues were obtained from a variance-covariance matrix. The degree of contribution of each morphometric character was determined by correlations between the original variables and the scores (loadings). The principal component analysis, "shearing method" (Humphries et al., 1981) and the multivariate allometric coefficients (Jolicoeur, 1963) were calculated in order to characterize differences in shape by size. We used a *t*-test to compare mean values between two morphometric characters of the same species (Sokal & Rohlf, 1981).

Hyla elianeae n.sp.

(fig. 1-2)

Holotype. MNRJ 17297, adult male, collected at Bela Vista (ca. 22°06'S, 56°31'W), 180 m above sea level, State of Mato Grosso do Sul, Brazil, between 20 and 23 January 1967, by S. G. Nunes, S. T. Albuquerque, J. Jim and E. Izecksohn.

Paratopotypes. Fifty-six adult males and five adult females (MNRJ 17209, 17226-17234, EI 8884-8934), collected with the holotype.

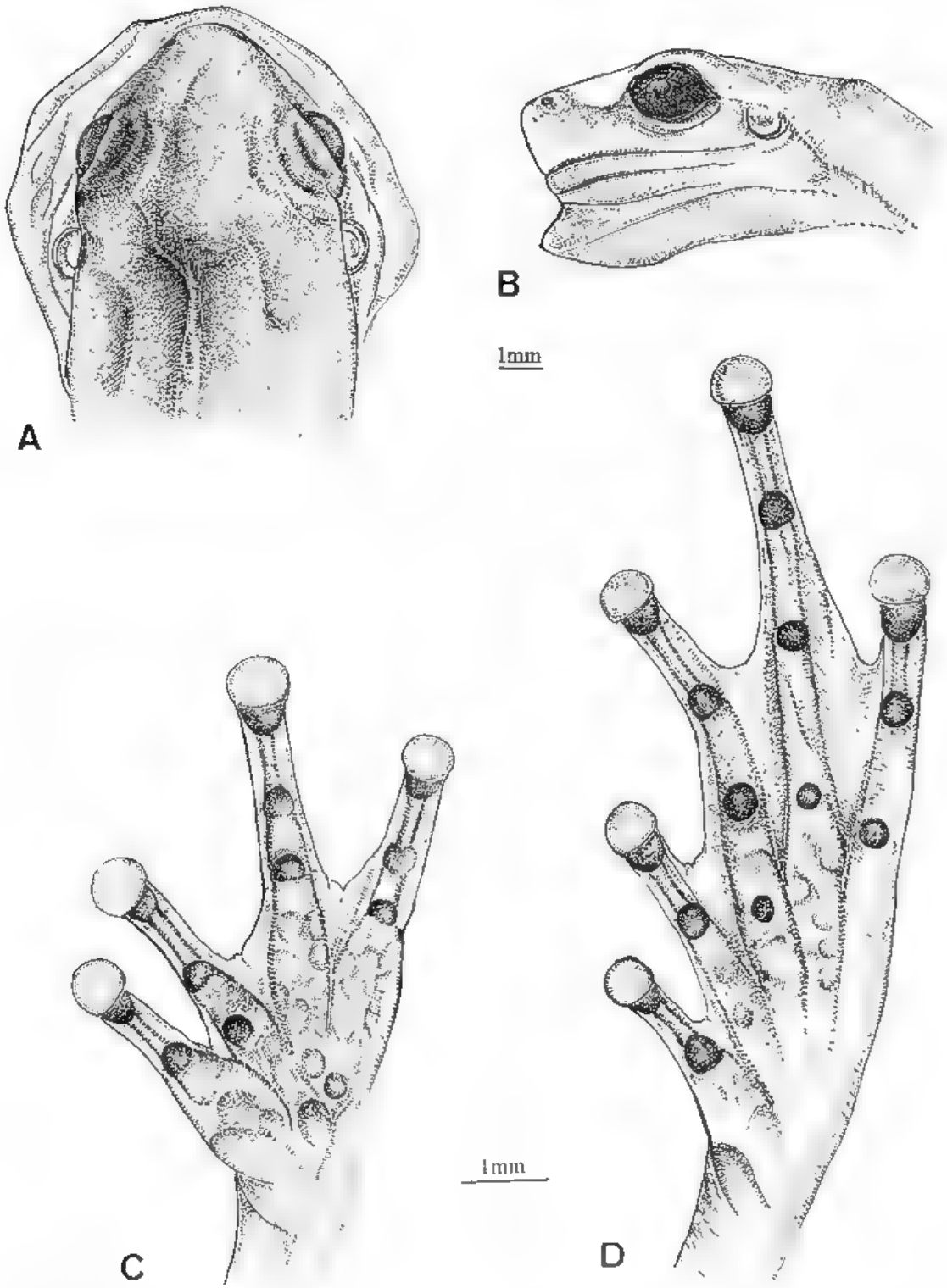

Fig. 1. *Hyla clarae*, MNRJ 17297, holotype. (A) Dorsal and (B) lateral views of head; ventral views of (C) hand and (D) foot.

Fig. 2 – Dorsal views of adult males of *Hyla chanchae* (A) MNRJ 17204, Botucatu São Paulo, (B) MNRJ 17296 Cachoeira do Marimbondo São Paulo (C) MNRJ 17297, holotype, Bela Vista, Mato Grosso do Sul

Diagnosis. – Species characterized by the following combination of traits: (1) in life, dorsum consistently green, (2) in preservative, dorsal surfaces pink to violet, (3) snout-vent length 20.0-25.5 mm SVL in males and 25.0-26.0 mm in females, (4) dorsum immaculate, (5) body robust, (6) lateral limits of dorsum under the lower border of tympanum (fig. 3B1), (7) canthus rostralis rounded (fig. 1A-B)

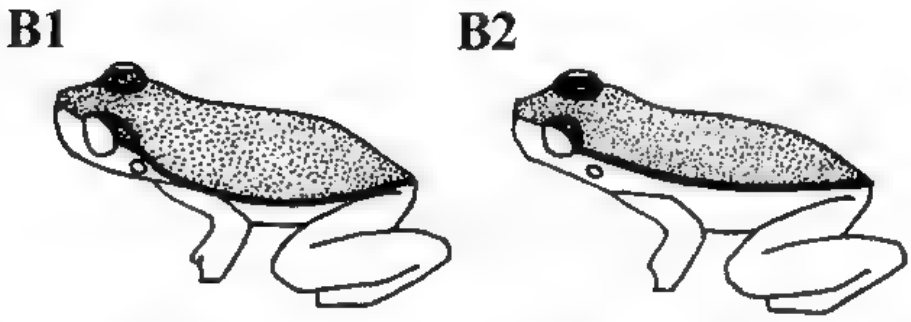

Fig. 3 – Standards for lateral limits of dorsum. (B1) under the lower border of tympanum, (B2) above the upper border of tympanum. Pattern B1 occurs in *H. elianeae*, while pattern B2 occurs in the other species of the *H. rubicundula* group.

The absence of a single mid-dorsal sacral stripe in *Hyla elianeae* distinguishes this species from *H. tritaeniata*, *H. araguaya* and *H. cerradensis*, which have such a pattern. No specimen of *H. elianeae* has two anterior divergent dorsal brown stripes fused with two brown sacral stripes, while many individuals of *H. anataliasiasi* have such a pattern. Furthermore, a broad and irregular dorsolateral stripe, with or without an upper white pinkish stripe is present in some specimens of *H. elianeae*, distinguishing this species from *H. anataliasiasi*, which has thin and well marked stripes. The lateral limits of dorsum in *H. elianeae* are under the lower border of the tympanum (fig. 3B1), while *H. anataliasiasi*, *H. cachimbo*, *H. tritaeniata*, *H. araguaya*, *H. cerradensis* and the specimens of *H. rubicundula* from State of Minas Gerais always have these above the tympanum (fig. 3B2). The absence in *H. elianeae* of a thin white stripe on the edges of tibia, above a thin brown stripe, distinguishes it from *H. rubicundula*, *H. anataliasiasi*, *H. tritaeniata*, *H. araguaya* and *H. cerradensis*, which may have these stripes. The presence in *H. elianeae* of a broad white pinkish stripe above a brown canthal stripe distinguishes it from *H. anataliasiasi*, *H. tritaeniata*, *H. araguaya* and *H. cerradensis*, which have the canthus well defined by a thin white stripe above a brown stripe. The head of *H. elianeae* is wider than long, its width contained about 3.4 times in the SVL, distinguishing it from *H. anataliasiasi*, which has the head longer than wide, its width contained about 3.6 times in the SVL. The snout in *H. elianeae* is rounded, truncate or slightly acuminate, while in *H. cachimbo* it is strongly protruding. The canthus rostralis in specimens of *H. elianeae* from central Brazil and northern State of São Paulo is rounded and not well defined. The canthus rostralis of other species of the *H. rubicundula* group is flat and well defined. The robust body and the maximum SVL in *H. elianeae* (20.0-25.5 mm in males, 25.0-26.0 mm in females) distinguish this species from *H. cachimbo* (19.8-21.0 mm in males; 24.2 mm in female), *H. rubicundula* (18.0-23.4 mm in males; 21.6-25.1 mm in females), *H. anataliasiasi* (16.0-21.8 mm in males, 16.6-21.6 mm in females), *H. tritaeniata* (17.6-20.9 mm, 20.6-21.5 mm in females), *H. araguaya* (18.9-20.5 mm in males) and *H. cerradensis* (18.9-19.3 mm in males).

Description. This description is based on specimens from Bela Vista, Mato Grosso do Sul only, which is the best sampled and most morphologically homogeneous population. The other geographic samples will be characterized within the geographic variation section. Descriptive statistics for the type series are furnished in tab. 1.

Table 1 - Descriptive statistics of morphometric characters for *Hyla elianeae* from central Brazil *n*, number of specimens for which data are available, *x*, arithmetic mean, *min*, minimum value, *max*, maximum value, *s*, standard deviation, *CV*, coefficient of variation

Characters	Males						Females ($n = 5$)				
	n	*x*	*min*	*max*	*s*	*CV*	*x*	*min*	*max*	*s*	*CV*
SVL	66	23 59	20 7	25 5	1 15	4 89	25 75	25 4	25 9	0 18	0 72
HW	66	6 99	6 2	7 6	0 31	4 47	7 58	7 4	7.7	0 12	1 58
HL	66	6 87	6 1	7 3	0 25	3 70	7 47	7 3	7 6	0 15	2 09
ED	66	2 49	2 1	2 8	0 13	5 27	2 63	2 5	2 7	0 08	3 18
UEW	62	1 60	1 2	1 8	0 12	7 76	1 37	1 2	1 5	0 14	10 49
IOD	62	2 14	1 8	2 9	0 21	9 94	2 58	2.5	2.7	0 14	5 58
END	66	1 71	1 5	1 9	0 11	6 58	1 80	1 7	1 8	0 05	2 77
IND	66	1 57	1 4	1 9	0 09	6 33	1 66	1 5	1 7	0 08	5 38
THL	66	10 92	9 2	12 2	0 59	5 47	12 05	11 8	12 3	0 20	1 71
TL	66	10 94	9 4	12 5	0 62	5 68	12 11	11 7	12 6	0 36	2 98
TD	65	1 08	0 8	1 4	0 10	9 60	1 25	1 1	1 5	0 14	11 31
NSD	66	1 20	1 0	1 4	0 07	6 24	1 22	1 1	1 3	0 08	6 85
UAR	65	6 33	5 5	7 3	0 38	6 15	6 58	5 7	7 2	0 66	10 03
FAR	65	4 11	3 3	5 3	0 38	9 35	4 18	3 8	4 6	0 33	7 91
HAL	65	6 49	5 6	7 6	0 39	6 12	7 10	6 6	7.6	0 42	5 95
3FD	66	0 97	0 7	1 8	0 16	17 11	1 03	0 9	1 1	0 09	9 46
FL	65	16 06	14 0	17 9	0 85	5 31	17 71	17 0	18 3	0 51	2 90
4TD	65	0 85	0 6	1 0	0 07	9 05	1 00	0 8	1 1	0 10	10 00

Body robust, head wider than long ($t = 2\ 46$, $n = 66$, $P < 0\ 0001$); internarial distance smaller than eye-nostril distance ($t = 7\ 95$, $n = 66$, $P < 0.0001$) and eye diameter ($t = 45\ 55$, $n = 66$, $P < 0.0001$), eye diameter greater than eye-nostril distance ($t = 36\ 66$, $n = 66$, $P < 0\ 0001$), snout truncate, acuminate or rounded in dorsal outline, and truncate or rounded in lateral outline, nostrils slightly protuberant, directed laterally or slightly forward, sometimes located on small raised areas, making the internarial region appear furrowed; canthus rostralis rounded, not well defined, loreal region oblique; eyes slightly or moderately prominent, tympanum distinct, nearly circular; supratympanic fold sometimes present, covering partially the tympanum, vocal sac single, subgular, tongue cordiform or ovoid, vomerine teeth present in two patches between choanae, irregular in shape and position. Forearm more robust than arm, shorter ($t = 32.86$, $n = 65$, $P < 0\ 0001$), hands with a distinct palmar tubercle (fig. 1C), prepollex distinct, subarticular tubercles rounded; distal tubercle of third and fourth fingers sometimes bifid; supernumerary tubercles present, third finger disk diameter greater than fourth toe disk ($t = 4\ 92$, $n = 65$, $P < 0\ 00001$), modal webbing formula I 2 - 2+ II 2+

Table 2 Descriptive statistical tables of morphometric characters for *Hyla elianeae* from southern São Paulo (morphotype SSP) and northern São Paulo (morphotype NSP). *n*, number of specimens for which data are available, *x*, arithmetic mean, *min*, minimum value, *max*, maximum value, *s*, standard deviation; *CV*, coefficient of variation

Characters	Morphotype SSP							Morphotype NSP						
	Males						Female ($n = 1$)	Males						Female ($n = 1$)
	n	*x*	*min*	*max*	*s*	*CV*		*n*	*x*	*min*	*max*	*s*	*CV*	
SVL	46	22 87	20.1	25 1	1 05	4 59	25 1	23	22 93	21.5	25.5	1 02	4 46	25 8
HW	46	6 72	6.0	7.3	0.32	4.79	6 9	23	6.78	6.4	7.7	0.34	5 14	7 2
HL	46	6 56	6 0	7.1	0 25	3.82	7 4	23	6 76	6.7	6 2	7.35	3 98	7 1
ED	46	2 37	2.0	2 7	0 11	4.71	2 7	23	2.57	2 2	3 0	0.17	6 87	2 5
UEW	44	1 57	1.2	1 9	0 15	10 03	1 5	23	1 59	1.2	2 1	0.15	10 02	1.7
IOD	44	2 23	1 8	2 6	0 14	6 65	2 5	22	2 27	1.7	2 7	0 18	7 93	2 3
END	46	1.70	1 4	2 0	0 13	7.74	1.7	23	1 58	1 4	1 8	0 12	7.98	1 6
IND	46	1 56	1.4	1 8	0 08	5 22	1 7	23	1 50	1 4	1.7	0 07	4 92	1 5
THL	44	11 02	9 6	11 9	0 57	5 18	11.4	22	10 54	9 4	11 5	0 59	5 65	11 4
TL	46	11 09	9.6	12.5	0.56	5.13	11.7	23	10 92	9.9	11.9	0.53	4 91	11 4
TD	46	0 99	0 8	1.3	0.09	9.40	1.2	22	1 00	0 8	1.2	0.10	10 60	1 2
NSD	46	1.19	1 0	1 5	0 11	9 45	1.2	23	1 13	1 0	1.2	0 07	6 20	1 1
UAR	46	6 28	5.6	6 8	0 28	4 55	6 8	23	6 43	5 7	7 1	0 37	5 86	7 0
FAR	46	4 11	3.6	4 9	0 25	6 08	4 3	23	4 11	3 4	4 9	0 40	9 84	3 9
HAL	46	6 51	5 4	7.2	0 40	6 22	6 5	23	6 24	5 6	7 2	0 39	6 27	6 7
3FD	46	0 86	0 5	1 0	0 10	11 85	0 9	23	0 96	0.8	1 2	0.09	9 53	1 0
FL	46	16 17	14 2	18 2	0 89	5 53	17 0	23	15 74	14.3	17 2	0.84	5.35	16 7
4TD	44	0 82	0 5	1 1	0 11	14 54	0 8	22	0 86	0.6	1 1	0.12	13 98	1 0

Table 3. Frequency of dorsal head shape patterns among morphotypes SSP, NSP and CBR. The patterns are illustrated in fig. 4. Numbers in parentheses indicate the percentage of the state for each morphotype. *n*, number of specimens for which data are available, *, specimens considered juveniles or subadults

Morphotypes	*n*	A1	A2	A3	A4	A5	A6
SSP	49	21 (43)	14 (28)	13 (26)			1 (2)
NSP	37		4 (10)*		10 (27)	23 (62)	
CBR	66		20 (30)				46 (69)

– 3 ¼ III 3 – 2 ¼ IV. Legs moderately robust; femur and tibia approximately same size and length ($t = 0.17$, $n = 66$, $P < 0.87$); sum of femur and tibia lengths smaller than SVL ($t = 8.57$, $n = 66$, $P < 0.0001$), foot with subarticular tubercles always rounded (fig. 1D); supernumerary tubercles variable in shape and number; toe disks slender, modal webbing formula, I 1 ½ – 2 ¼ II 1⁺ – 2 ¼ III 1 ½ – 2 ¼ IV 2 ½ – 1⁺ V.

Color in preservative. Dorsum reddish to pinkish brown, immaculate; canthus rostralis, although not well defined, sometimes delimited inferiorly by a brown stripe; loreal region with a variable degree of melanization, a dorsolateral brown stripe sometimes present on the flanks, extending from the posterior corner of orbit to near the groin, sometimes bordered above by a white pinkish stripe, thigh light brown, immaculate, a brown stripe sometimes present on the anterior and posterior edges of the upper surface of tibia, in addition to random dorsal dark brown dots. Ventral surfaces immaculate buff.

Measurements of holotype. SVL 25.5; HW 7.5; HL 7.3; ED 2.4; UEW 1.6; IOD 2.0, END 1.8, IND 1.7; THL 11.5, TL 12.2; TD 1.1; NSD 1.3, LAR 6.7, FAR 4.3, HAL 7.3, 3FD 0.9, FL 17.9; 4TD 0.8.

Geographic variation. Three main geographic morphotypes were diagnosed (fig. 2), and were named and coded as follows (each entry in coded series contains the code, the code name, the number of specimens analyzed, and the localities where the samples came from): (1) SSP, "southern São Paulo", *n* = 58 (Minas Gerais: Belo Horizonte; São Paulo: Botucatu, Campinas, Corumbataí, Itapeva, Jaguariúna, Paulínia, Pindamonhangaba, Pirassununga and Sumaré). (2) NSP, "northern São Paulo", *n* = 37 (São Paulo: Icém, Indiaporã and Pirassununga). (3) CBR, "central Brazil", *n* = 72 (Goiás: Rio Verde; Mato Grosso: Caceres; Mato Grosso do Sul: Bela Vista and Estância Caiman). Descriptive statistics of each morphotype are furnished in tab. 1-2.

The specimens from southern São Paulo (morphotype SSP) differ from the type specimens (morphotype CBR) by the presence of the following characteristics: dorsum sometimes purple and sometimes with additional melanization, absence of a lateral white pinkish stripe, snout profile sometimes protruding; canthus rostralis distinct, dorsal head shape patterns A1-A3 (fig. 4; tab. 3); modal palmar webbing formula, I 2 ½ – 2 ½ II 2 – 2 ¼ III 3 – 2⁺ IV; legs slender; modal plantar webbing formula, I 1 ¼ – 2⁺ II 1⁺ – 2 ¼ III 1 ½ – 2⁺ IV 2⁺ – 1⁺ V.

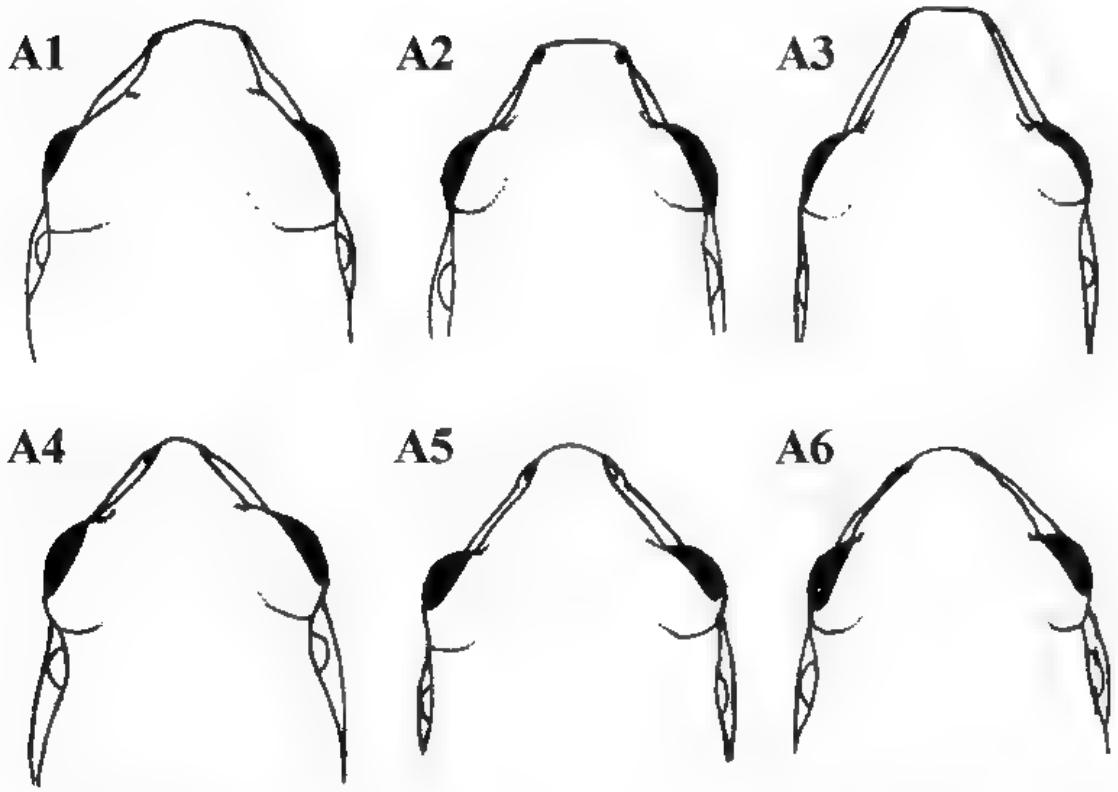

Fig. 4 - Standards for dorsal head shape patterns. (A1) and (A3) southern São Paulo (morphotype SSP), (A4) and (A5) northern São Paulo (morphotype NSP), (A6) central Brazil (morphotype CBR), pattern (A2) occurs among the smallest specimens within the three morphotypes, including adults and juveniles.

The specimens from northern São Paulo (morphotype NSP) differ from the type specimens (morphotype CBR) by the presence of the following characteristics: dorsum with additional melanization, head as long as wide ($t = 0.62$, $n = 23$, $P = 0.53$), snout acuminate or truncate in dorsal outline, rounded or slightly truncate in lateral outline, canthus rostralis distinct and rounded, eyes very prominent, dorsal head shape patterns A4-A5 (fig. 4, tab. 3), prepollex slightly developed; modal webbing formulae, I 2 ¼ – 2 ¾ II 2 – 3 ¼ III 3 – 2 ¼ IV, legs slender, femur and tibia approximately with the same size, femur longer than tibia ($t = 2.18$, $n = 22$, $P = 0.03$), supernumerary tubercles indistinct, modal plantar webbing formulae, I 1 ¼ – 2 ¼ II 1^+ — 2 ¼ III 1 ¼ – 2 ¼ IV $2^+ - 1^+$ V.

Multiple discriminant function analysis was used to analyze morphological variation among morphotypes SSP, NSP and CBR, using 18 measurement variables (fig. 5). There was no statistical difference among mean values of SVL for the three morphotypes analyzed, which implies similar "size" among the samples. Two significant canonical axes (Wilks $P < 0.05$) resulted from this analysis, representing 100 % of the total variation. Both canonical vectors alternated positive and negative coefficient values (tab. 4A), which implies variation in "shape" (HUMPHRIES et al., 1981). However, SVL had the fourth largest significant loading in

Tab.e 4 A. standardized coefficients and loadings from discriminant function analysis for morphometric characters of combined samples from morphotypes SSP, NSP and CBR (fig 5) B standardized coefficients from principal component analysis ("shearing method") of combined samples areas of morphotype SSP (fig 8A) C, standardized coefficients and loadings from discriminant function analysis for morphometric characters of the combined geographic samples of morphotypes SSP and NSP (fig 9) D, standardized coefficients from principal component analysis ("shearing method") of combined dorsal head shape patterns A2 and A6 of morphotype CBR (fig 10A)

r Pearson correlation coefficient of the original data with the scores obtained from the discriminant function analysis (loadings), ns, not significant, * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$

Characters	A				B		C				D	
	VCI	VCII	*r* (I)	*r* (II)	PCI	PCII	VCI	VCII	*r* (I)	*r* (II)	PCI	PCII
SVL	-0 36	-0 16	-0 35***	-0 03ns	0 22	0 11	0	0 79	0 28*	0 65***	0 25	-0 06
HW	-0 62	0 35	-0 45***	-0 01ns	0 21	0 11	-0 69	-0 19	0 13ns	0 64***	0 12	-0 02
HL	-0 61	0 60	-0 54***	0 21*	0 14	0 07	-0 39	0 22	-0 11ns	0 61***	0 08	-0 01
ED	-0 07	0 35	-0 27***	0 57***	0 07	0 01	-0 57	-0 14	-0 52***	0 38***	0 02	0 01
UEW	0 44	0 25	-0 03ns	0 11ns	0 51	0 26	0 13	1 02	0 11ns	0 65***	0 11	-0 02
IOD	0 40	0 26	0 30***	0 12ns	—	—	-0 16	0	-0 12ns	-0 06ns	0 11	-0 03
END	-0 07	-0 50	-0 20*	-0 51***	0 33	0 17	0 57	-0 16	0 66***	0 36***	0 27	-0 07
IND	-0 13	-0 48	-0 05ns	-0 36***	0 17	0 09	0 45	-0 83	0 61***	0 17ns	0 27	-0 07
THL	-0 82	-1 37	0 00ns	-0 37***	0 28	0 14	0 53	0 28	0 65***	0 44***	0 29	-0 07
TL	1 98	1 12	0 15ns	-0 15ns	0 28	0 15	-0 52	-0 37	0 46***	0 53***	0 15	-0 06
TD	-0 40	-0 30	0 52***	-0 05ns	0 06	0 03	-0 06	-0 45	-0 08ns	-0 07ns	0 21	-0 05
NSD	0 12	-0 08	-0 15ns	-0 31***	0 25	0 10	0 20	0 36	0 51***	0 40***	0 26	-0 06
UAR	0 24	0 35	0 01ns	0 20*	0 11	0 02	0 13	0 51	-0 11ns	0 52***	0 20	0 00
FAR	-0 03	-0 19	0 06ns	0 00ns	0 21	0 11	-0 08	0 42	0 18ns	0 45***	0 25	-0 06
HAL	0 06	-0 34	-0 04ns	-0 32***	0 27	0 12	0 40	0 62	0 62***	0 47***	0 25	-0 06
3FD	-0 39	-0 01	-0 28***	0 29***	0 08	-0 15	-0 76	0 09	-0 37***	0 39***	0 46	-0 17
FL	0 00	-0 06	0 04ns	-0 24**	0 28	0 15	0 47	-0 69	0 54***	0 51***	0 22	-0 05
4TD	0 37	0 06	-0 00ns	0 14	0 12	-0 16	0 70	-0 21	-0 05ns	0 32**	0 14	-0 03

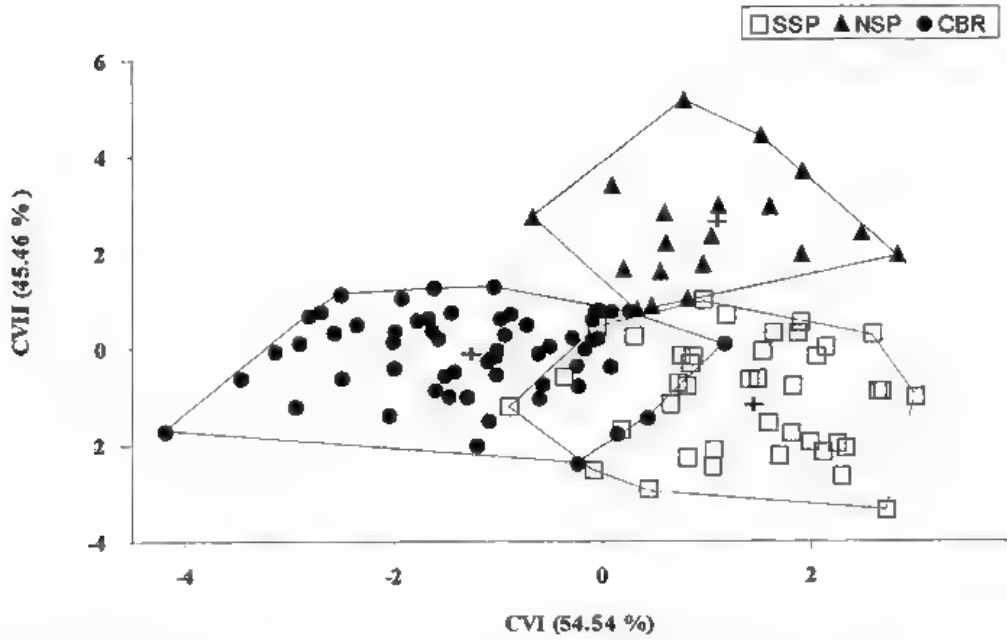

Fig. 5 - Projection of individual scores resulted from the multiple discriminant function analysis for 18 morphometric characters of the combined samples of morphotypes SSP, NSP and CBR in the space of the first with the second canonical axes.

for dorsal head shape patterns in both directions. In order to analyze the patterns of morphological variance between the morphotypes NSP and SSP, we combined them in a single discriminant function analysis, joining some geographic samples, based on geographic proximity and morphological similarity, to increase the number of specimens in the groups analyzed. Only two statistically significant canonical vectors were obtained (Wilks $P < 0.05$), and the scores were projected in the space of these two axes (fig. 9). The coefficients from the first and second canonical axis have both positive and negative values (tab. 4C), representing "shape" (Humphries et al., 1981). The first axis discriminates the morphotype SSP from NSP. The second axis partially discriminates the geographic samples of each morphotype, and in morphotype SSP the discrimination follows exactly a latitudinal gradient throughout the "cerrado" corridor of São Paulo. Moreover, SVL has the largest loading in the second axis (tab. 4C), which implies that the "size" strongly contributes for the discrimination. Note that the samples of morphotypes SSP and NSP from Pirassununga are morphologically very similar. This analysis supports our previous conclusion that there is interbreeding between morphotypes SSP and NSP, stated by the directional cline for dorsal head shape patterns in both directions, and that SSP and NSP belong to the same species, *Hyla elianeae*.

Variation within CBR – Three geographic samples of morphotype CBR were examined (fig. 6): Bela Vista and Estância Caiman, State of Mato Grosso do Sul, Cáceres, State of Mato Grosso. The samples from Mato Grosso do Sul were combined based on geographic proximity, morphological similarity and because Estância Caiman sample has only four specimens.

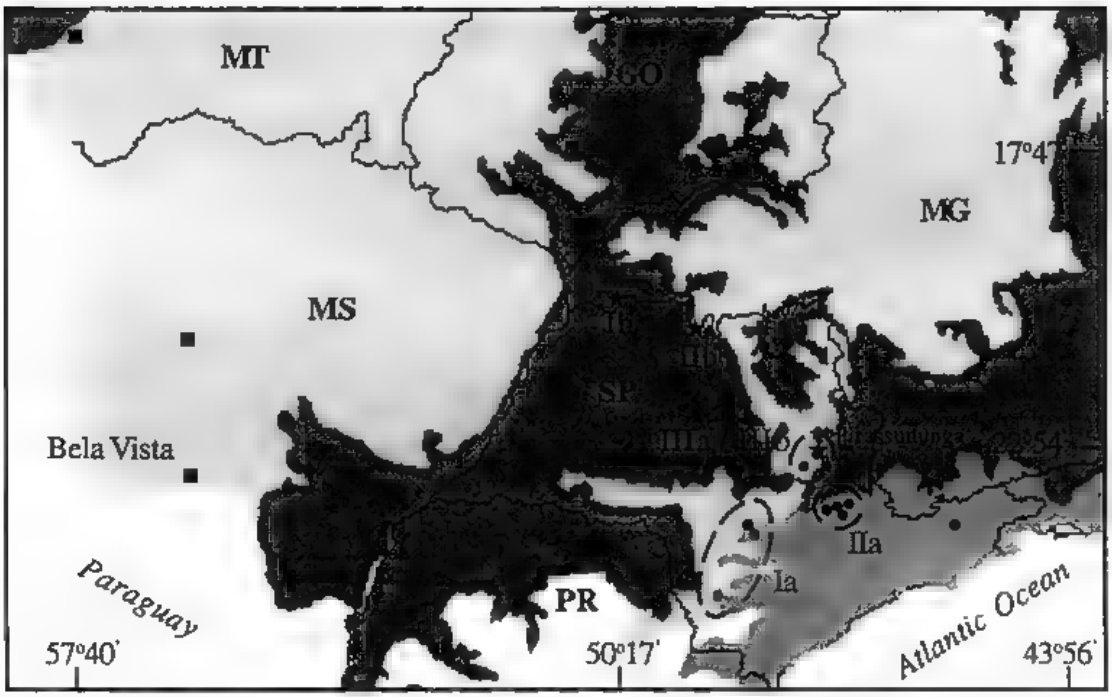

Fig 6 Geographic distribution of morphotypes SSP (circles), NSP (stars) and CBR (squares) of *Hyla elianeae* in Brazil In order to analyze morphological variation, nine geographic samples of morphotype SSP throughout the "cerrado" corridor of São Paulo were grouped into three areas (Ia, IIa and IIIa) based on geographic proximity and morphological similarity The samples of morphotype NSP were named Ib, IIb and IIIb following the same criteria Shadings represent, from darker to lighter, respectively deciduous tropical forest, Atlantic forest, and "cerrado" GO, State of Goias, MG, State of Minas Gerais, MS, State of Mato Grosso do Sul, MT, State of Mato Grosso, PR, State of Paraná; SP, State of São Paulo

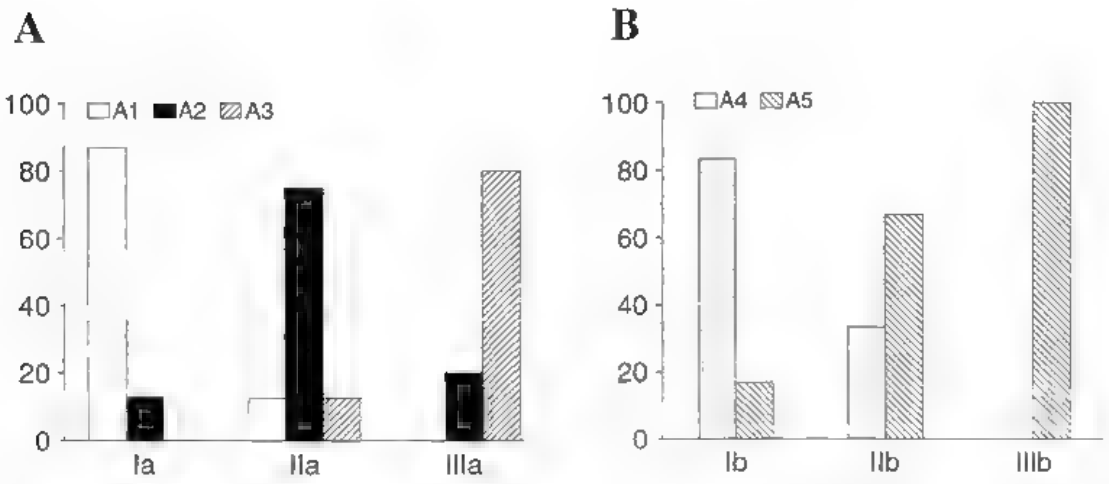

Fig 7 Frequency in percentage, of dorsal head shape patterns obtained in (A) morphotype SSP, and (B) morphotype NSP for areas delimited in fig 6. Standards of each pattern are in fig 4

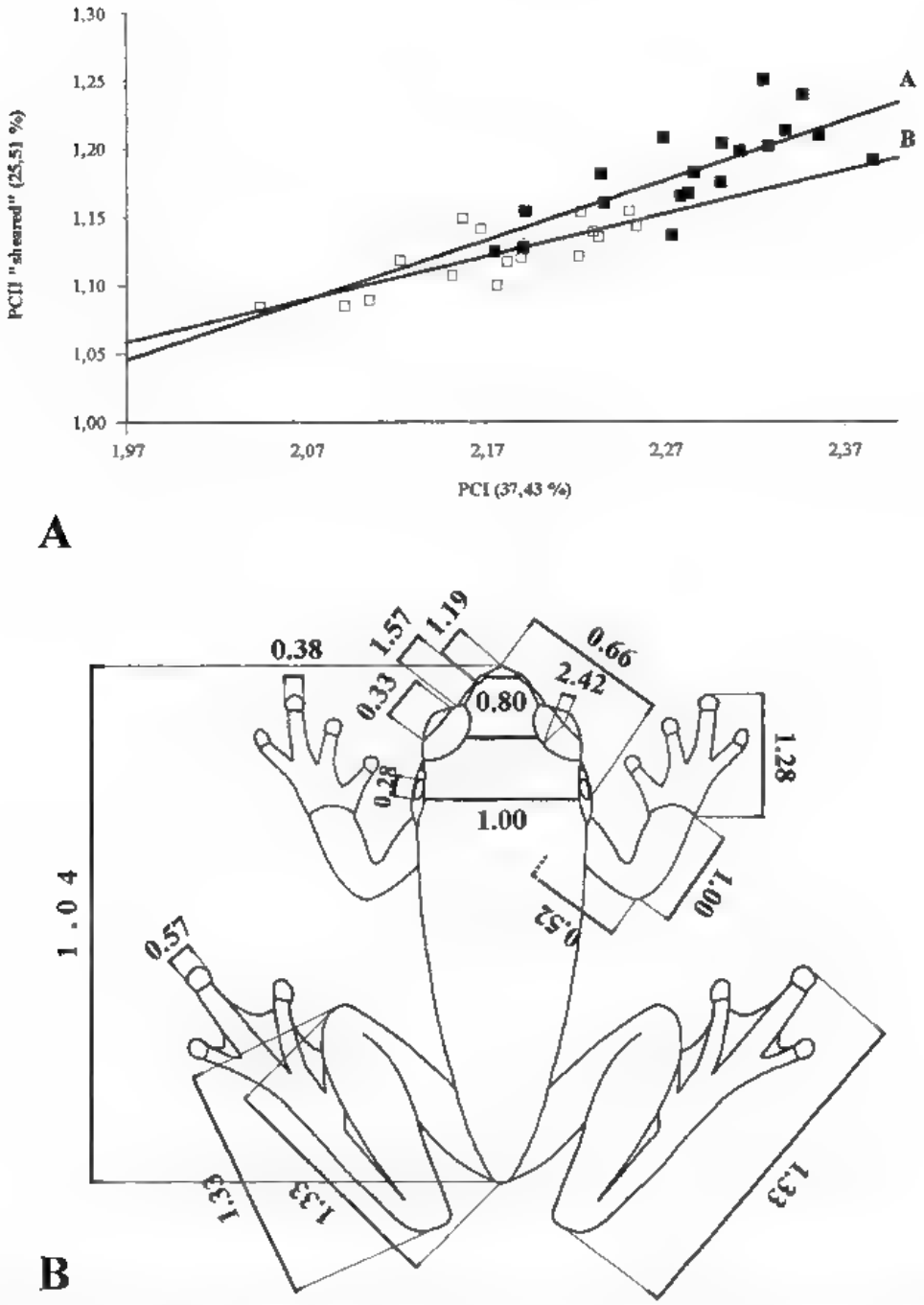

Fig. 8 — (A) Projection of individual scores resulting from the principal component analysis, using the "shearing method" (HUMPHRIES et al., 1981), of 17 morphometric characters for the combined sample areas Ia (close squares) and IIa/IIIa (open squares) from morphotype SSP (fig. 6). A and B are regression lines of the individual scores of the second component by the first, respectively, for sample areas Ia and IIa/IIIa (ontogenetic trajectories). Both regressions are statistically significant for $P < 0.01$. (B) Allometric multivariate coefficients for combined sample areas Ia, IIa and IIIa of morphotype SSP. A value lower than 1 indicates negative allometry, higher than 1, positive allometry; equal to 1, an isometric character.

The analysis of the dorsal head shape patterns showed differences in degree and kind between the two samples. Specimens from Cáceres have only character A6, while specimens from Bela Vista/Estância Caiman have characters A2 and A6. The pattern A6 occurs in the largest specimens and A2 in the smallest ones. In order to verify if the occurrence of three kinds of dorsal head shape patterns is caused by ontogenetic changes, we analyzed the combined patterns A6 (largest specimens) and A2 (smallest specimens) by the principal component analysis, using the "shearing method" (HUMPHRIES et al., 1981). Individual scores were projected in the space of the first two principal components (fig. 10A). The first axis has all coefficients with positive values (variation in "size") and the second has coefficients with both positive and negative values (tab. 4D), denoting variation in "shape" (HUMPHRIES et al., 1981). The result denotes a clear variation in "shape" by "size", that is, the successive changes from pattern A2 to A6. The different contribution of each character measured can be estimated by multivariate allometric coefficients (JOLICOEUR, 1963), which allow us to project the changes in shape over size increments (fig. 10B).

Comparing these coefficients with those obtained from morphotype SSP (fig. 8B), we may observe that variables END, UEW, NSD, TD and UAR are the most different. The first three variables reflect the main differences in head shape between these two morphotypes, where morphotype SSP has adult specimens with longer snouts, less prominent eyes and more truncate snouts than morphotype CBR.

However, it is questionable if the differences observed in dorsal head shape (fig. 4) are correlated with different size distributions available for the three geographic morphotypes. In order to use an appropriate measure of size, the first principal component for each morphotype was extracted. The correlations (Pearson) of the scores of each first principal component with dorsal head shape patterns observed in each morphotype were calculated. The morphotypes SSP, NSP and CBR had statistical significant correlations between size and dorsal head shape patterns (respectively, $r = 0.58$, $n = 38$, $P < 0.0001$, $r = 0.55$, $n = 50$, $P < 0.0001$; $r = -0.43$, $n = 27$, $P < 0.02$). Within the three morphotypes, pattern A2 occurs in the smallest specimens, and patterns A1, A4, A5 and A6 occur in the largest specimens. Thus, the smallest individuals of the three morphotypes have similar dorsal head shape (pattern A2), that become different over size increments. This fact explains the partial superposition of morphotypes SSP and CBR and the best discrimination for morphotype NSP observed in fig. 5, where morphotype NSP has less small individuals than morphotypes SSP and CBR. This result also explains the morphological proximity of morphotypes SSP and NSP within geographic samples where both have the smallest specimens (fig. 9).

Geographic remarks and taxonomic conclusions. Some geographic considerations may aid the understanding of the morphological diversity of the samples under study. The species of the *Hyla rubicundula* group occur only in "cerrado" formations and are never found in tropical rainforests. In the State of São Paulo, "cerrado" formations occur from the boundaries of Botucatu to the north of the state, throughout a strait band in Depressão Periférica Paulista ("cerrado" corridor, fig. 6). These areas are discontinuous islands surrounded by "cuestas" and scarps covered by tropical rainforests. This fact suggests a certain geographic isolation among these populations and may explain the morphological variation observed. The "cerrado" corridor seems to act as the only possible path for interaction

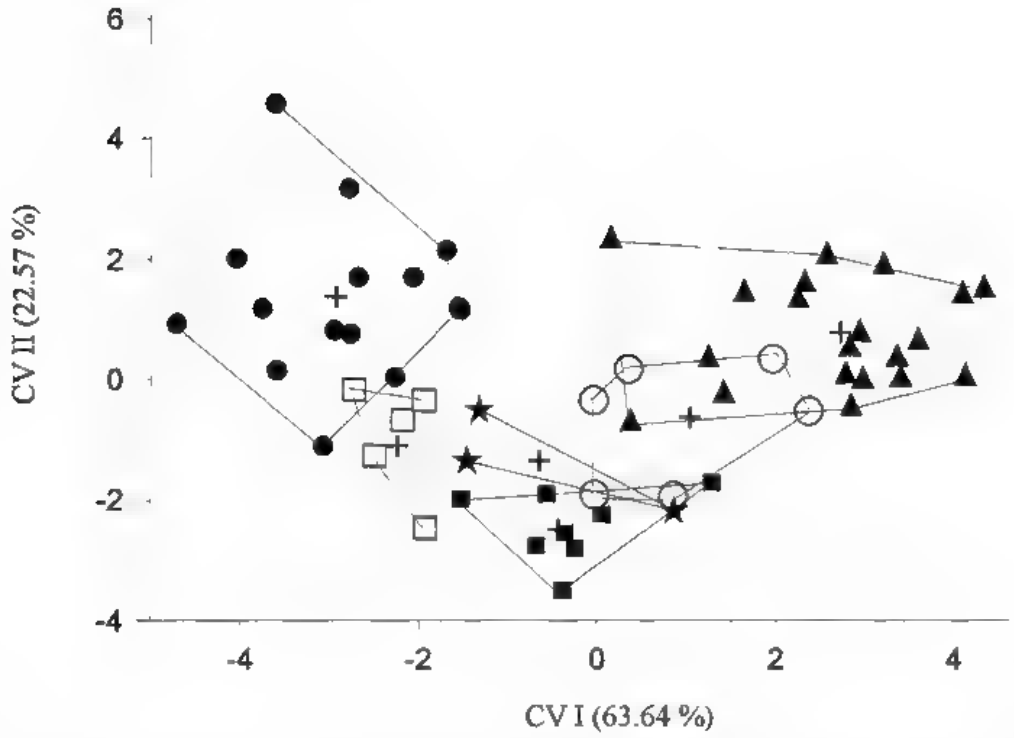

Fig. 9 Projection of individual scores resulting from the multiple discriminant function analysis for 18 morphometric characters of the combined geographic samples of morphotypes SSP and NSP in the space of the first with the second canonical axes. Some samples had to be combined in order to increase the number of specimens under study. Morphotype NSP: Indiaporã + Icém (closed circles) and Pirassununga (open squares), morphotype SSP: Botucatu + Pardinho + Itapeva (triangles), Paulínia + Campinas (open circles), Corumbataí (stars) and Pirassununga (closed squares). Note that the second axis, in which SVL had the largest contribution, partially discriminates the geographic samples of each morphotype and, in morphotype SSP the discrimination follows exactly a latitudinal gradient throughout the "cerrado" corridor (fig. 6), also, the NSP sample from Pirassununga and SSP samples from Pirassununga and Corumbataí are morphologically very similar, supporting the hypothesis of interbreeding between the two morphotypes throughout the "cerrado" corridor.

between São Paulo samples with those from central Brazil. This hypothesis explains why all specimens examined or cited in the literature were from localities surrounding or inside the corridor.

The external morphology and morphometrics do not allow us to consider each morphotype as a different species. The coloration patterns vary among morphotypes mainly by degree, not kind. The dorsal head shape varies in kind, but is related to morphological variation throughout geographical gradients or allometric changes. A dorsal head shape pattern is in some cases intermediate between two standards, making it quite impossible to assign to a discrete class. Moreover, the presence of certain dorsal head shape patterns in areas not contiguous with other populations to where these are common may indicate that interbreeding exists. In a sample from Itapety, São Paulo (Serra da Mantiqueira), one

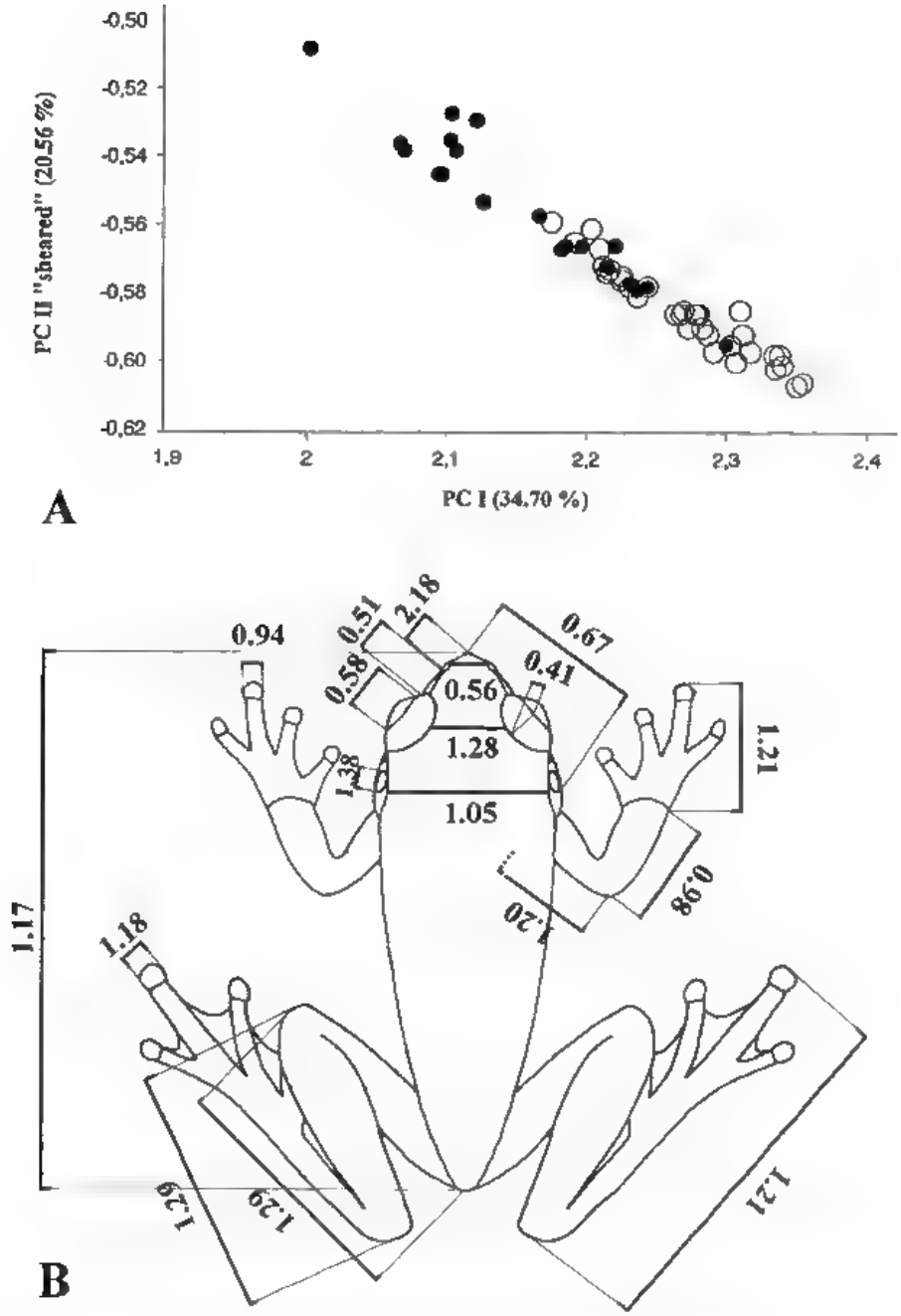

Fig. 10 (A) Projection of individual scores resulting from the principal component analysis, using the "shearing method" (HUMPHRIES et al., 1981), of 18 morphometric characters for the combined dorsal head shape patterns A8 + A9 (open circles) and A10 (closed circles) from Bela Vista, Mato Grosso do Sul (morphotype CBR). (B) Allometric multivariate coefficients for the entire Bela Vista sample. A value lower than 1 indicates negative allometry, higher than 1, positive allometry, equal to 1, an isometric character.

specimen has dorsal head shape pattern A6, which is characteristic of morphotype CBR from central Brazil. One specimen from Rio Verde, Goias, has dorsal head shape pattern A3, otherwise only observed in morphotype SSP. According to the "cerrado" corridor that joins the samples under study, the patterns for the morph from Goiás should be similar to that obtained from northeastern São Paulo (morphotype NSP) or from central Brazil (morphotype CBR), instead of a pattern from southern São Paulo.

Natural history. Jim (1980) stated that the specimens he identified as *Hyla rubicundula*, which we name *H. elianeae*, occupy upper parts of grass or herbaceous vegetation (0.5-1.0 m high), associated to permanent or temporary ponds formed by rainwater in open habitats. According to this author, the specimens from Bela Vista, Mato Grosso do Sul, occupy a different position in vegetation, but the same environment.

Distribution. – *Hyla elianeae* occurs in the states of Goiás, Mato Grosso, Mato Grosso do Sul and São Paulo (fig. 6). One specimen was recorded from Belo Horizonte, Minas Gerais. It also may occur in the chacoan region of Paraguay, which is contiguous with the Brazilian cerrado, as stated by Frost (1985) for *Hyla rubicundula*. These regions all belong to the Cerrado Domain (Ab'Saber, 1977).

Etymology. The name is a noun in the genitive case honoring Eliane de Freitas Napoli, the first author's wife.

Additional specimens examined

Hyla araguaya. BRAZIL. Goias: Santa Rita do Araguaia (paratypes, MZUSP 66719-66721). Mato Grosso. Alto Araguaia (holotype, MZUSP 66803, paratypes, MZUSP 66796-66802, 66804-66806; MNRJ 17240-17241).

Hyla cachimbo. BRAZIL. Pará: Cachimbo, Serra do Cachimbo (holotype, MZUSP 21912, paratypes, MZUSP 21910-21911, 21913-21918, 21920-21926; MNRJ 17298-17299).

Hyla cerradensis. BRAZIL. Mato Grosso do Sul: Ribas do Rio Pardo (holotype, ZUEC 06733; paratypes, ZUEC 06734-06737; MNRJ 17293).

Hyla elianeae. BRAZIL. Goiás: Rio Verde (MZUSP 33873). Mato Grosso: Cáceres (MNRJ 17194-17199). Mato Grosso do Sul: Bela Vista (MNRJ 17301), Estância Caiman (MZUSP 64955-64958). Minas Gerais: Belo Horizonte (MNRJ 17213). São Paulo: Botucatu (MNRJ 17192-17193, 17203-17209, JJ 2425-2430, 2434-2445, MZUSP 3863), Campinas (MNRJ 20880); Corumbataí (CFBH 2081, 2083-2084), Iceni, Cachoeira do Marimbondo (MNRJ 17156-17159, 17166, 17296, MZUSP 34648, DZSJRP 01324-01325, 02302-02307), Indiaporã (MNRJ 17160-17165), Itapeva (MZUSP 60862-60863), Jaguariuna (ZUEC 8166); Paulínia (CFBH 237-239, MNRJ 20877-20879), Pindamonhangaba (MNRJ 17200-17202), Pirassununga (MZUSP 2451-2452, 3023, 3028-3029, 3055, 3058, 3064, 3068, 3076, 9114-9115, 14434-14436, 14438-14443, 14445-14447, 34465-34469, 54423, MNRJ 1208), Sumaré (ZUEC 8385).

Hyla rubicundula. BRAZIL. BAHIA: Barreiras (MNRJ 0933-0940, 0946, 6145-6154); Jupaguá (MNRJ 0943-0944). MINAS GERAIS. Alfenas (MNRJ 17126-17134); Andrequicé (MNRJ 17110), Arinos (MZUSP 64500-64504); Barão de Cocais (MNRJ 17210-17212); Belo Horizonte (MNRJ 17214-17220, MZUSP 519, 34647); Buritis (MZUSP 64449-64452, 64455-64458, 64460-64464), Buritizeiro (MNRJ 17111-17116), Esmeraldas (ZUEC 4023); Jaboticatubas (MZUSP 57712-57713); Januária (MNRJ 0942); Lagoa Formosa (MNRJ 17123); Lagoa Santa (MNRJ 0947, 3081, 6155-6177, 13287, 17117-17121, 17124-17125; MZUSP 34012-34023; ZUEC 4150), Manga (MNRJ 0941), Pimenta (MNRJ 17319-17321); Pirapora (MNRJ 17101-17109); Uberlândia (MNRJ 17305-17308); Unaí (MZUSP 64386, 64389-64392, 64396; 64398-64402; MNRJ 17135); Vespasiano (MNRJ 17221-17223; MZUSP 12691-12693). GOIÁS. Aragarças (MZUSP 20983), Cavalcante (MZUSP 66543, 66570, 66574, 66576,); Cristalina (MZUSP 64522); Goiânia (MNRJ 17136-17155; 17300); Iaciara (MZUSP 66527-66528); Monte Alegre de Goiás (MZUSP 66403-66407, 66450, 66456), Nova Roma (MZUSP 66358-66360), Porangatu (MNRJ 17167-17168), Santa Rita do Araguaia (MZUSP 66650-66654); São Domingos (MZUSP 66597-66603); escarpa da Serra Dourada (ZUEC 7505). PIAUÍ: Uruçuí (MNRJ 17224).

Hyla anataliasiasi. – BRAZIL. MATO GROSSO: Posto Diauarum (MZUSP 49588-49617), Posto Leonardo (MZUSP 49339-49393)

Hyla tritaeniata BRAZIL. MATO GROSSO: São Vicente (paratypes, MNRJ 13986, 14205, WCAB 16220). MATO GROSSO DO SUL. Sonora (UFMT 2147-2150)

Hyla sp. aff *tritaeniata* BRAZIL. MATO GROSSO DO SUL Campo Grande (MNRJ 17235-17239); Costa Rica (DZSJRP 02836), Iguatemi (MZUSP 33807) MINAS GERAIS: Ribeirão São Pedro (MNRJ 17248-17251); São Roque de Minas (ZUEC 4349, 7174-7176); Unaí (MNRJ 17247) SÃO PAULO: Botucatu (JJ 1625-1629, 3685-3713); Brotas (CFBH 412-416); Itatinga (DZSJRP 1841-1843, 1845), Itapeva (MZUSP 60866), Pirajú (MNRJ 17225), Pirassununga (MNRJ 17242-17246, MZUSP 14450-14471, 30983-30984, 9100-9113), São Paulo (MZUSP 60924)

RESUMEN

Una nueva especie de *Hyla* del grupo de *H. rubicundula* es descrita de Bela Vista, Estado de Mato Grosso do Sul, Brasil. La nueva especie vive en los ambientes de los cerrados de las regiones central y sudeste de Brasil, poseendo tres formas geográficas principales, que se diferencian por sus formas y tamaños diferentes. Cada variación intra- y interpopulacional es caracterizada para determinar com precision los límites de la especie. La análisis fue basada en la morfologia externa y en la morfometría. La nueva *Hyla* es caracterizada por presentar mayor tamaño en su grupo, cuerpo robusto, limite lateral del dorso abajo del bordo inferior del timpano, dorso inmaculado y canthus rostralis redondeado.

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